Everted vesicae of the *Timandra griseata* group: methodology and differential features (Geometridae, Sterrhinae)

Pasi Sihvonen

Finnish Museum of Natural History, Department of Entomology, University of Helsinki, P.O. Box 17 (P. Rautatiekatu 13), FIN-00014 Helsinki, Finland; e-mail: pasi_sihvonen@hotmail.com

Summary. The internal male genitalia of *Timandra griseata* Petersen, 1902, *T. comai* Schmidt, 1931 and *T. recompta* (Prout, 1930) are illustrated for the first time with the vesica being everted. The vesicae have loosely species-specific, somewhat variable characters that can be interpreted to have anatomical correspondences with the junction of the female corpus bursae and appendix bursae of the bursa copulatrix. Full evertion of these membranous structures is difficult and their interpretation for identification purposes should be performed cautiously. The vesica evertion technique using the lumen of an injection needle is described.

Zusammenfassung. Die inneren männlichen Genitalstrukturen von *Timandra griseata* Petersen, 1902, *T. comai* Schmidt, 1931 und *T. recompta* (Prout, 1930) werden erstmalig mit ausgestülpter Vesica abgebildet. Die Vesicae zeigen schwach artspezifische, aber etwas variable Ausprägung, die eine anatomische Korrespondenz zum Übergang des Corpus bursae in den Appendix bursae im weiblichen Genital aufweisen. Die vollständige Ausstülpung der membranösen Strukturen ist schwierig, die Interpretation im Zuge von Artidentifikationen sollte mit Vorsicht erfolgen. Die Technik der Vesicaausstülpung mittels einer Injektionsnadel wird beschrieben.

Resumé. L'armure génitale mâle interne de *Timandra griseata* Petersen, 1902, *T. comai* Schmidt, 1931 et de *T. recompta* (Prout, 1930) est illustrée pour la première fois avec les vesicae évaginées. Les vesicae offrent des caractères quelque peu variables et ainsi faiblement diagnostiques au niveau spécifique, pouvant être interprétés comme étant sujets à une correspondence anatomique avec la jonction de la corpus bursae et de l'appendix bursae de la bursa copulatrix chez la femelle. L'évagination complète de ces structures membraneuses est difficile et leur interprétation à des fins d'identification doit être effectuée avec circonspection. La technique d'évagination de la vesica au moyen d'une épingle à seringue est décrite.

Key words. Geometridae, *Timandra*, vesica, methodology.

Introduction

Although the method of vesica evertion has been available for over half a century (Hardwick 1950; redefined and illustrated in Lafontaine & Mikkola 1987), these structures are still rarely illustrated in taxonomical works on Lepidoptera, except for the Noctuidae where this technique has become routine (e.g. Fibiger 1997). The structures of the vesica can enable understanding of mating mechanisms (Callahan & Chapin 1960) and may allow better resolution of particular species-level problems (e.g. Kerppola & Mikkola 1987; Lafontaine *et al.* 1987; Fibiger 1990). Thus far, smaller moths such as geometrids have largely been left unnoticed with regard to the evertion technique (but see e.g. Holloway 1993; Troubridge 1997). Recently Dang (1993) introduced two methods to study these structures in smaller species such as Tortricidae and Nepticulidae.

The *Timandra griseata* group has been revised (Kaila & Albrecht 1994) to include three species: *T. griseata* Petersen, 1902, *T. comai* Schmidt, 1931 and *T. recompta* (Prout, 1930). These species are similar in external appearance, though identifiable. The interspecific differences in both male and female external genitalia are small. This

is the case especially for the male genitalia of *T. griseata* and *T. comai*. Earlier attempts to evert the male vesicae have been unsuccessful and it has therefore been unclear whether they could provide diagnostic features.

Here I will present that in the *Timandra griseata* group the everted male vesicae are loosely species-specific, i.e. with small intraspecific variation, and that they can be useful aids to species identification if carefully used. The male vesicae are shown to have species-specific structures that anatomically correspond with the female genitalia. In addition, I describe a modification of the vesica evertion technique that I have used successfully in many geometrid species.

Material and method of vesica evertion

Material examined. – *Timandra griseata*: Finland, Ta: Orivesi, [no date] ♂ (PS594); Al: Lemland, 16.7.1951 ♀; N: Helsinki, 16.7.1958 1♂ (PS596); Ka: Virolahti, 20.–26.8.1990 2♂ (PS597, PS609); Om: Ylivieska, 15.7.1971 ♂ (PS598); Oa: Korsnäs, 25.6.1934 ♂ (PS604); St: Pori, 21.6.1970 ♂ (PS606); St: Reposaari, 21.7.1969 ♀ and Tb: Jyväskylä, 24.6.[19]20 ♂ (PS611). – *Timandra comai*: Finland, N: Helsinki, 27.8.1965 and 5.9.1965 ♂ (PS595, PS599); N: Hanko, 16.8.1985 ♀; Ka: Virolahti, 4.–6.8.1990, ♂ (PS600), 3.–6.9.1990 ♂ (PS607), 1.–3.8.1989 1♀; N: Porvoo, 27.8.1951 1♂ (PS605); Al: Lemland, 19.6.1968 1♂ (PS610); Czech Republic, Bohemia, Teplice, ♂ [no date] (PS601) and Russia, Mari, El,12.6.1997 ♂ (PS612). – *Timandra recompta* ssp. *ovidius* Bryk, 1942: Russia, Kurils, 11.–18.9.1997 3♂ (PS602, PS603, PS608). – *Timandra recompta* ssp. *recompta* Prout: China, Heilongjiang, Fenglin State Natural Reserve, 48°05'N 128°80'E, 28.6.–10.7.2000 ♂ (PS614). All studied specimens are deposited at the Zoological Museum, University of Helsinki (ZMH).

The genitalia were dissected following the routine techniques described by Hardwick (1950), and terminology is according to Klots (1970). Most of the genitalia are stored in glycerol tubes attached to the specimens. Below I describe a vesica evertion method and it is assumed that the reader is familiar with the techniques for the preparation of the genitalia of Lepidoptera.

For the evertion of male vesica, I have modified the technique described by Dang (1993) so that the aedeagus is placed into the lumen of the needle and the vesica is everted through the incised caecum (fig. 1). This technique has been used earlier by K. B. Bolte (pers. comm.) and K. Mikkola (pers. comm.), so I claim no originality but describe the technique in detail in order to help other colleagues in their work. With this technique it is possible to evert vesicae from aedeagi which measure as little as 0.04 mm in diameter (personal observation).

1. Orientation and removal of the aedeagus. Before removing the aedeagus from the diaphragma, one should study the orientation of the aedeagus carefully. Especially the anterior opening of the aedeagus (the passage through which the ductus ejaculatorius enters the aedeagus) is of importance, since it can be used to orientate the genitalia so that the copulation posture can be reconstructed later. The aedeagus is removed from the diaphragm by gripping it gently with forceps at the caecum and pulling it carefully anteriorly or posteriorly. The aedeagus is placed into a dissecting dish containing 5% aqueous ethanol solution.

2. Removal of the caecum. The aedeagus should be stained (e.g. in Chlorazol Black) to demonstrate the position of the vesica and ductus ejaculatorius within the aedeagus. If such a structure lies within the caecum, it should be moved towards the distal open-

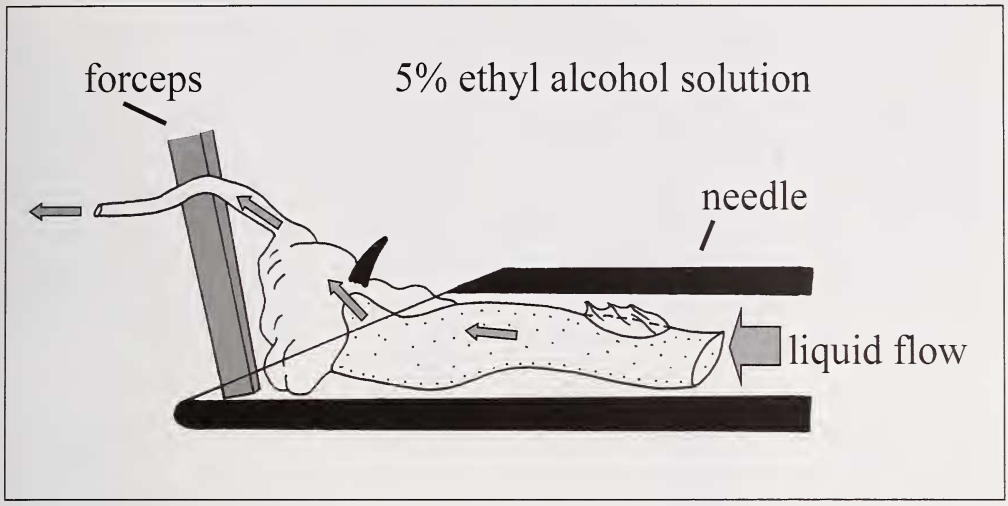

Fig. 1. The position of the aedeagus within the needle during the evertion of vesica. The aedeagus is held in place with the help of small forceps.

ing of the aedeagus using a small hair, needle or similar by pushing it gently from the anterior opening of the aedeagus. Once certain that the caecum is empty, it can be cut away using microscissors. The excess of the ductus ejaculatorius, if still outside the aedeagus, should be trimmed as short as possible. A long ductus ejaculatorius may get stuck within the aedeagus and obstruct the jet fluid which everts the vesica.

3. Pushing the vesica posteriorly. Through the opening of the caecum the inverted vesica is pushed to the posterior opening of the aedeagus with the help of a hair or similar tool. The use of sharp or strong tools which may easily puncture the membranous structures should be avoided.

4. Determining the needle size. The diameter of the needle lumen should be slightly wider than the maximum diameter of the aedeagus. Too small a needle may cause structures to break and too large a needle may allow the aedeagus to twist during the evertion and prevent the flow of the liquid. In this study I used a 27G (lumen diameter 0.19 mm) needle for laterally slightly bent aedeagi of approximately 0.14 mm in diameter. I have successfully used a 30G (lumen diameter 0.14 mm) needle for species with an aedeagus diameter of between 0.04–0.08 mm. The tip of the needle should be smoothed with emery paper so that it does not have sharp edges, see Dang (1993).

5. Evertion of the vesica. Fill the syringe with a mild solution of Chlorazol Black or 5% aqueous ethanol solution and fit it with a needle. Press the needle sidewise against the bottom of the dish and pass the aedeagus into the lumen of the needle except for the posterior end. Do not pass the aedeagus too far into the needle, as the walls of the needle may obstruct the full evertion of the vesica. Place the tip of the small forceps at the mouth of the needle to prevent movement of the aedeagus (Figure 1). Then apply moderate pressure to the plunger of the syringe until the vesica is fully everted and then maintain a steady flow until the vesica is adequately stained. Remove the forceps from

the tip of the needle and let the aedeagus fall into the dish. Transfer the aedeagus to a dish that contains 99.5% ethyl alcohol.

6. Fixing the structures. Fill the syringe with 99.5% ethanol or isopropanol and evert the vesica again as described above, to ensure that the membranous structures are fixed at their maximal size. Usually a steady flow of about 30 seconds is adequate. Leave the aedeagus in 99.5% ethanol or in absolute isopropanol for a few hours. It can later be safely transferred to glycerine for detailed study or mounted in euparal.

This technique is applicaple to females, too. For instance *Scopula frigidaria* Möschler (Geometridae, Sterrhinae) has a cup-like ostium bursae that can be placed into the lumen of the needle (personal observation).

Key to the species of *Timandra griseata* group based on vesica structures

The species recognition of *Timandra griseata* and *T. comai* on the basis of this character alone is highly susceptible to incorrect interpretation due to the difficulties in the preparation technique and should therefore be used cautiously.

1.	Diverticulum on ventral side covers the apex of the aedeagus	*recompta* (Fig. 2)
–	Diverticulum on ventral side does not cover the apex of the aedeagus	2
2.	Edge of the lateral diverticulum forms an angle of about 120°–130°	*griseata* (Fig. 2)
–	Edge of the lateral diverticulum forms an angle of about 90°–100°	*comai* (Fig. 2)

The vesicae of the *Timandra griseata* group

Timandra griseata (Fig 2). – Aedeagus about 1.7 mm long, 0.14 mm wide, slightly curved laterally, well sclerotized, surface smooth, apex round, slightly expanded dorsally near apex; anterior opening of aedeagus located dorso-laterally at approximately one-fifth of its length from anterior end; caecum slightly curved dorsally. Vesica larger than aedeagus, ventrally directed large diverticulum subapically, about two-fifth of length of aedeagus, oval, not covering apex of aedeagus, near apex laterally two small diverticula on opposite sides, edge of one with an angle of about 120°–130°, other one slightly sclerotized on posterior side on most specimens; near apex one large cornutus with small teeth dorsally, most often directed dorsolaterally; distal end of vesica directed posterolaterally, slightly turned laterally near opening of primary gonopore, narrowing distally, surface with shallow longitudinal grooves basally; primary gonopore opens from distal end of vesica.

Timandra comai (Fig. 2). – As in *T. griseata*, with the following small differences: anterior opening of aedeagus located dorsally; edge of one lateral diverticulum forming an angle of about 90°–100° in most specimens; direction of cornutus more variable.

Timandra recompta (Fig. 2). – As in *T. griseata*, with the following small differences: anterior opening of aedeagus located dorsally at around one-eight of its length from anterior end; subapical ventral diverticulum slightly covering apex of aedeagus; one lateral diverticulum large, edge of other one with an angle of about 100°; cornutus often directed ventrally.

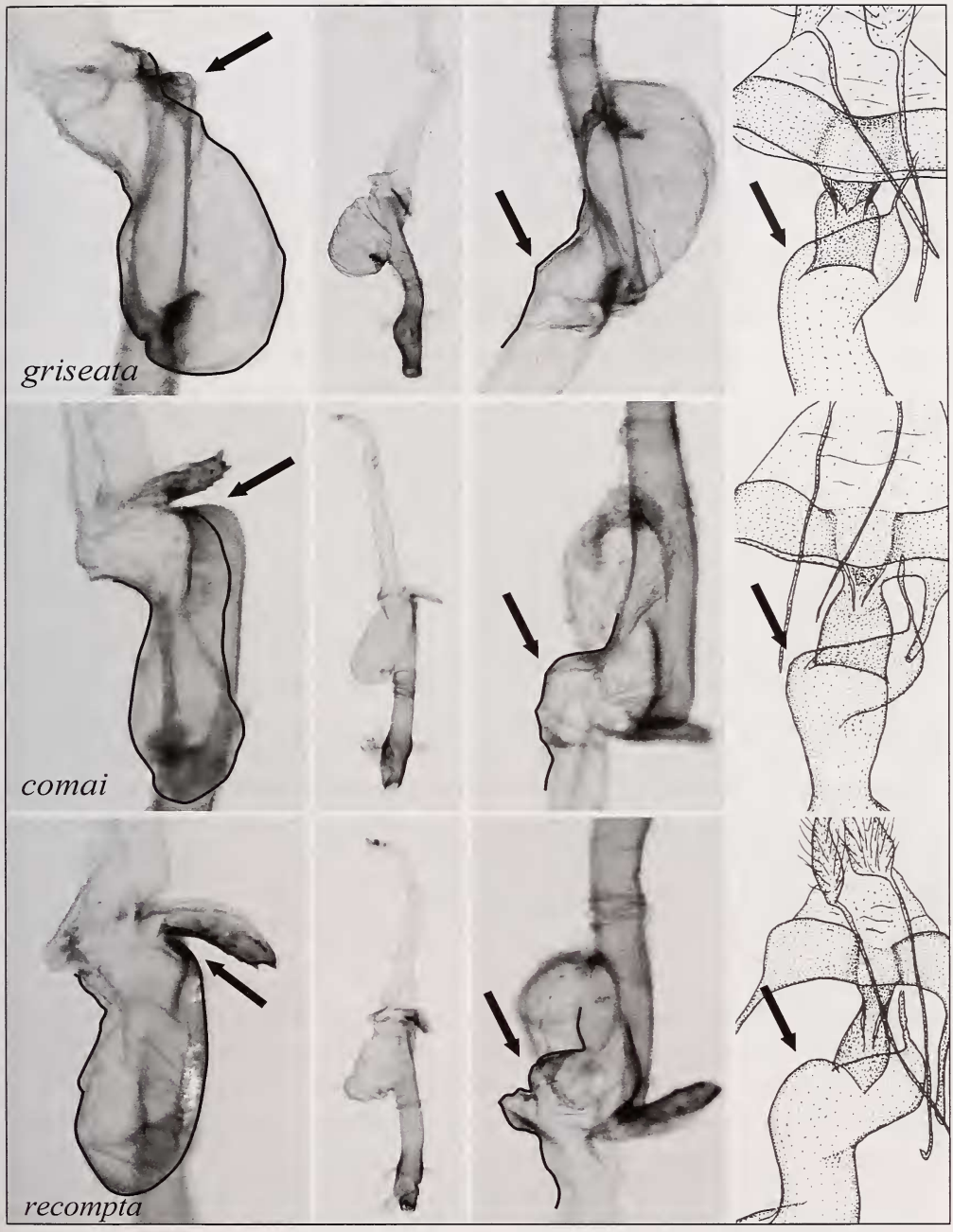

Fig. 2. The male aedeagus with everted vesica and female ductus bursae of the *Timandra griseata* group. Male *T. griseata* in lateral (from left), ventral and ventrolateral view (slide PS606) and female ductus bursae. Male *T. comai* in lateral (PS607), ventral (PS612) and ventrolateral (PS607) view and female ductus bursae. Male *T. recompta* in lateral, ventral and ventrolateral view (PS608) and female ductus bursae. In ventrolateral view the anterior end of aedegus is at the top of the figure, in order to show the aedeagus in the copulation posture relative to the female genitalia. The edges of the male membranous structures have been highlighted to show details. Illustrations of female genitalia are taken from Kaila and Albrecht (1994) with permission.

Discussion

The vesicae of species of the *Timandra griseata* group are very similar and they do not offer taxonomic characters that can be easily used for species identification. Nevertheless, the detailed differences that were found on the lateral diverticulum of the vesica, i.e. shape and angle, appear to be anatomically compatible with the junction of the corpus bursae and appendix bursae of the female genitalia (Fig. 2; Kaila & Albrecht 1994: Fig. 13). The phenomenon of species-specific genitalia is widespread among animals with intromittent genitalia (e.g. Eberhard 1985) as well as species-specific anatomical congruence in internal male and female genitalia (e.g. Callahan & Chapin 1960; Mikkola 1992; Sota & Kubota 1998). Thus the observed species-specific male characters of the *Timandra griseata* group that are anatomically compatible with the female genitalia, should not be considered only as being an artefact of the preparation technique.

According to this study *griseata*, *comai* and *recompta* have loosely species-specific structures in the internal genitalia, thus giving morphological support to the conclusion of Kaila and Albrecht (1994) to treat them as valid species, although some intraspecific variation is present. Due to the membranous nature of these structures in both sexes, their value as diagnostic characters are dependent upon preparation technique and therefore should be used cautiously.

To allow for possible geographical variation, specimens were dissected from Central Finland where *Timandra griseata* occurs alone (i.e. in allopatry to *T. comai*), from southern Finland, where *T. griseata* and *T. comai* occur sympatrically, and from Central Europe, where *T. comai* occurs in allopatry. Minor variation is present in the angle of the diverticulum as well as in other details of the vesica structures. It is possible that some of this variation is a result of the preparation of membranous structures. In a few cases, the identification of a particular specimen to a certain species was difficult if the decision was based on one character only. However, the combination of various vesica characters, i.e. the orientation of vesica, the position of the subapical ventral diverticulum in relation to the apex and the direction of the anterior opening of the aedeagus always resulted in a confident identification.

The difference in the direction of the anterior opening of the aedeagus was found to be constant, dorsolateral in *Timandra griseata* and dorsal in *T. comai* and *T. recompta*. Again, the use of this character is susceptible to incorrect interpretation, as the removal of the genitalia from the abdomen may distort the orientation of the aedeagus and lead to erroneous conclusions. The orientation of the vesica in relation to the rest of the genitalia does not differ between species, despite the difference in the orientation of the anterior opening of aedeagus.

This method for evertion of the vesicae has both advantages and disadvantages. Evertion through the caecum allows a strong and direct flow of liquid into the vesica and it usually effectively everts the diverticula, which may be difficult to evert with other techniques. In the *Timandra griseata* group, the subapical ventral diverticulum lies in close contact with the ventral wall of the aedeagus when the vesica is inverted. I was initially unable to evert the ventral diverticulum through the anterior opening of

the aedeagus, as it lies perpendicularly to the longitudinal axis of the aedeagus and the direction of flow was apparently not strong enough. All attempts through the caecum were successful, however, if the aedeagus was not pushed too far inside the lumen of the needle, allowing it to expand.

The most obvious advantage that comes from placing the aedeagus into the lumen of the needle is that one can evert much smaller vesicae as compared to inserting the needle into the aedeagus. Also, larger needles are easier to keep clean.

An obvious defect of the method is that part of the aedeagus is removed, which may cause other structures, namely the ductus ejaculatorius or vesica, to break. In addition, one needs to be very careful not to loose the caecum during the preparation.

Acknowledgements

I wish to thank K. Mikkola for teaching me the techniques of vesica evertion. K. Fiedler, A. Hausmann and L. Kaila are thanked for critical comments and discussions on the earlier version of this manuscript. I am grateful to S. Venn for revising my English.

References

Callahan, P. S. & J. B. Chapin 1960. Morphology of the reproductive systems and mating in two representative members of the family Noctuidae. *Pseudaletia unipuncta* and *Peridroma margaritosa*. with comparison to *Heliothis zea*.– Ann. ent. Soc. Am. **53**: 763–782.

Dang, P. T. 1993. Vesicas of selected tortricid and small lepidopterous species, with descriptions of new techniques of vesica evertion (Lepidoptera: Tortricidae, Oecophoridae, Gelechiidae, and Nepticulidae).– Can. Ent. **125**: 785–799.

Eberhard, W. G. 1985. Sexual selection and animal genitalia.– Harvard University Press, Cambridge. 244 pp.

Fibiger, M. 1990. Noctuidae Europaeae. Vol. **1**. Noctuinae I.– Entomological Press, Sorø. 565 p.

Fibiger, M. 1997. Noctuidae Europaeae. Vol. **3**. Noctuinae III.– Entomological Press, Sorø. 418 p.

Hardwick, D. F. 1950. Preparation of slide mounts of Lepidopterous genitalia.– Can. Ent. **82**: 231–235.

Holloway, J. D. 1993. The moths of Borneo. Family Geometridae, Ennominae.– Mal. Nat. J. **47**: 1-309, +18 pls.+593 figures.

Kaila, L. & A. Albrecht 1994. The classification of the *Timandra griseata* group (Lepidoptera: Geometridae, Sterrhinae).– Ent. scand. **25**: 461–479.

Kerppola, S. & K. Mikkola 1987. *Autographa buraetica* (Staudinger), a plusiine moth new to Finland and Europe (Lepidoptera, Noctuidae).– Notulae Ent. **67**: 119–123.

Klots, A. B. 1970. 20. Lepidoptera.– *In*: Tuxen, S. L. (ed.), Taxonomists glossary of genitalia in insects.– Munksgaard, Copenhagen: 115–130.

Lafontaine, J. D. & K. Mikkola 1987. Lås-och-nyckel systemen i de inre genitalierna av Noctuidae (Lepidoptera) som taxonomiska kännetecken.– Ent. Meddr. **55**: 161–167.

Lafontaine, J. D., K. Mikkola & V. Kononenko 1987. *Anarta cordigera* (Lepidoptera: Noctuidae: Hadeninae), a species complex.– Can. Ent. **119**: 931–940.

Mikkola, K. 1992. Evidence for lock-and-key mechanisms in the internal genitalia of the *Apamea* moths (Lepidoptera, Noctuidae).– Syst. Ent. **17**: 145–153.

Sota, T. & K. Kubota 1998. Genital lock-and-key as a selective agent against hybridization.– Evolution **52**: 1507–1513.

Troubridge, J. T. 1997. Revision of the Nearctic species of the genus *Entephria* Hübner (Lepidoptera: Geometridae, Larentiinae).– Ent. scand. **28**: 121–139.